Complete Listing of Claims Pursuant to 37 C.F.R. §1.121

Pursuant to 37 C.F.R. §1.121 the following is a complete listing of the claims of the present application. Claims 1-7 have been cancelled. Claims 8-28 are new.

1. [Cancelled]

2. [Cancelled]

3. [Cancelled]

4. [Cancelled]

5. [Cancelled]

6. [Cancelled]

7. [Cancelled]

8. [New] A method of purifying human recombinant α-L-iduronidase, or a biologically active fragment or mutein thereof, comprising the steps of

a) obtaining culture medium from a culture of Chinese Hamster Ovary (CHO) cells that have been transformed with a nucleic acid that encodes said human recombinant α-L-iduronidase;

b) adjusting the pH of the culture medium to an acidic pH;

c) subjecting said pH-adjusted medium to ultrafiltration;

d) subjecting the filtered medium produced by step (c) to a first dye-affinity chromatography purification step;

e) subjecting the eluant from step (d) to a first metal-ion chelate chromatography step;

f) subjecting the eluant from step (e) to a hydrophobic interaction chromatography (HIC) step; and

g) concentrating and diafiltering the eluant from step (f), to yield a purified preparation of purified human recombinant α-L-iduronidase which has a greater than

99% purity as determined by quantity of contaminating CHO protein per mg of total protein in said preparation.

9. [New] The method of claim 8, wherein said first dye-affinity chromatography purification step is performed on a Cibacron-Blue affinity chromatography matrix.

10. [new] The method of claim 8, wherein said first metal-ion chelate chromatography step is performed on a copper-chelating Sepharose FF matrix.

11. [New] The method of claim 8, wherein said HIC step is performed on a phenyl-Sepharose High Performance chromatography matrix,

12. [New] The method of claim 9, wherein said purification on said Cibacron-Blue dye interaction chromatography column produces a seven to ten fold purification of said human α-L-iduronidase as compared to the initial medium applied to said chromatography column.

13. [New] The method of claim 8, wherein said method comprises using 10% glycerol in all buffers to increase the quantitative recovery of said human α-L-iduronidase.

14. [New] The method of claim 8, wherein step (b) results in the pH of the fluid adjusted to pH 5.3.

15. [New] The method of claim 8, wherein said purified human recombinant α-L-iduronidase has a specific activity greater than 200,000 units per milligram protein.

16 [New] The method of claim 12, wherein said purified human recombinant α-L-iduronidase has a specific activity greater than 240,000 units per milligram protein.

17. [New] The method of claim 8, wherein said purified human recombinant α-L-iduronidase comprises one or more mannose-6-phosphate residues.

18. [New] The method of claim 17, wherein said purified human recombinant α-L-iduronidase comprises a mannose-6-phosphate residue attached at position 3 and a mannose-6-phosphate residue attached at position 6.

19. [New] The method of claim 8, wherein said purified human recombinant α-L-iduronidase has a half-life inside a cell of approximately 5 days.

20. [New] The method of claim 8, wherein said culture of CHO cells is a culture of cell line 2.131 CHO cells.

21. [New] The method of claim 8, wherein said CHO cells are cultured in a protein-free culture medium having a pH of between 6.8 and 7.0, said medium being supplemented with 7.6 mg/L thymidine, 13.6 mg/L hypoxanthine, 375μg/mL G418 and 5% fetal bovine serum

22 [New] The method of claim 21, wherein said CHO cells are grown to confluence at a density of between 2.0 x 105 to 2.5 x 105 cells per ml.

23. [New] The method of claim 23, wherein the medium of said cells at confluence is harvested for said purification of human recombinant α-L-iduronidase, or a biologically active fragment or mutein thereof.

24. [New] The method of claim 23, wherein said medium of said cells at confluence is harvested by continuous perfusion.

25. [New] The method of claim 25, wherein said continuous perfusion comprises exchanging between 2 to 3.5 culture volumes of said medium over 24 hours.

26. [New] The method of claim 22, wherein production of said human recombinant α-L-iduronidase is enhanced by supplementing said medium with sodium butyrate for 12 hours to induce α-L-iduronidase gene expression.

27. [New] The method of claim 26, wherein said sodium butyrate is removed from said medium 12 hours after initial induction with said sodium butyrate.

28. [New] The method of claim 27, wherein said production of human recombinant α-L-iduronidase is reinduced with sodium butyrate every 48 hours over a 21 day protein production period.